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Immunoglobulins in Bovine Mammary Secretions

QUANTITATIVE CHANGES IN EARLY LACTATION AND ABSORPTION BY THE NEONATAL CALF

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Summary. Antibodies against *Escherichia coli* 08 in bovine colostrum and serum have been studied by gel filtration chromatography and the red cell linked antigen–antiglobulin reaction. Immunoglobulins were assayed by radial immunodiffusion.

In bovine colostrum anti-*E. coli* activity was attributable to IgA and the level of activity was comparable with that detected in IgM and IgG fractions. The immunoglobulin profile and distribution of *E. coli* antibodies in post-colostral calf serum was similar to that in colostrum, thus providing an unusual occurrence of high levels of secretory IgA in serum. However the immunoglobulin disappeared rapidly from the serum with an apparent half life of approximately 2 days; the value for IgM was 4 days.

During the first 3 days of lactation the levels of immunoglobulins fall rapidly and milk is subsequently secreted with uniformly low levels of antibodies.

INTRODUCTION

The immunoglobulins of bovine colostrum and post-colostral calf serum have been the subject of numerous investigations which have been adequately reviewed by Butler (1969). Much of the emphasis is centred on the selective transfer of IgG1 to the colostrum and the non-selective intestinal absorption of colostral immunoglobulins by the calf. In recent studies a bovine colostral immunoglobulin analogous to human IgA has been characterized as well as secretory component (Mach, Pahud and Isliker, 1969; Porter and Noakes, 1970; Butler 1971). Vaerman (1970) has provided additional evidence for the classification of this immunoglobulin as IgA by demonstrating immunological cross-reactivity with the human counterpart. Quantitative studies of IgA in a range of bovine secretions now demonstrate that a secretory immune system mediated by IgA is operative in the bovine (Porter and Noakes 1970; Mach and Pahud 1971; Butler 1971).

The importance of colostral immunoglobulins to survival in the neonatal calf is well known and although the levels of *E. coli* antibodies in bovine colostrum and calf serum has been examined by Ingram and Malcomsen (1970) there is no information available on the relative contribution of the specific immunoglobulin classes to neonatal defence. Quantitative studies of immunoglobulins IgG and IgM in bovine colostrum and calf serum have been described (Klaus, Bennett and Jones 1969; Penhale and Christie, 1969) and attention has been drawn to the possible importance of IgM (Logan and Penhale, 1971). It is now pertinent to enquire about the importance of IgA.

In colostrum of other species such as the human (Adinolphi, Glynn, Lindsay and Milne, 1966) and the pig (Porter, 1969) IgA plays an important antibacterial role. Although secretory IgA does not persist at high concentrations throughout lactation and is unlikely to contribute to intestinal defence in the calf, it is present in relatively large amounts in the colostrum and is absorbed by the neonatal calf (Porter, 1971). In this paper the anti-globulin haemagglutination test is used to assess the anti-*E. coli* activity attributable to specific immunoglobulins in bovine colostrum, milk and calf serum.

MATERIALS AND METHODS

Chromatography

Gel filtration chromatography was carried out on Sephadex G-200 columns (45 × 2·5 cm and 90 × 2·5 cm) using 0·85 per cent NaCl in 0·1 M Tris-HCl buffer pH 7·2. Large scale gel filtration was carried out on Sephadex G-150 (80 × 6 cm).

Anion exchange chromatography was carried out on diethylaminoethyl cellulose as part of the procedure for isolation of immunoglobulins (Porter and Noakes, 1970).

Microelectrophoresis

Protein fractions were examined by immunoelectrophoresis in agar gels using antisera raised in New Zealand White rabbits. Electrophoresis in polyacrylamide gel was carried out according to the technique of Orstein and Davies (1964).

Isoelectric focusing

An electrofocusing column LKB 8101 was used having a capacity of 110 ml. Carrier ampholytes manufactured by LKB-Producteur AB, Bromma, Sweden, were used and separation was conducted according to LKB procedure applying approximately 10 mg of protein sample in a pH gradient 3–10.

Analytical ultracentrifugation

Ultracentrifugal analysis was carried out in a Beckman Model E centrifuge equipped with phase plate schlieren diaphram. Sedimentation coefficients were determined at 20° employing rotor speeds of 59,780 rev/min.

Isolation of immunoglobulins IgG1, IgG2 and IgM

Immunoglobulin IgG2 was prepared from a serum globulin fraction precipitated with 12 per cent Na_2SO_4 by chromatography on DEAE cellulose in 0·01 M phosphate buffer, pH 7·6. IgG2 was isolated in the fall through fraction and the 7S immunoglobulin was obtained after gel filtration on Sephadex G-200.

A euglobulin fraction precipitated after dialysis of serum against water was used as the starting material for preparation of IgM by gel filtration on Sephadex G-200.

Immunoglobulin IgG1 was prepared from bovine milk whey by chromatography on DEAE cellulose. The fraction eluted with 0·05 M NaCl in 0·01 M phosphate buffer pH 7·6 was subjected to gel filtration on Sephadex G-200 for selection of a suitable fraction containing 7S immunoglobulin.

The immunoglobulin fractions were further purified by electrophoresis in Sephadex G-25 at pH 8·6. The gel was mixed with 0·07 M barbiturate buffer and spread in a 5-mm layer on a Perspex plate (20 × 10 cm) contact with the electrode buffer was made with filter

paper wicks. The immunoglobulin fraction was absorbed into a filter paper strip inserted in a slot at the centre of the plate. After completion of electrophoresis at 120 V for 5 hours at 4°, the gel was divided into 1-cm sections and transferred to tubes for elution with 0·15 M NaCl. The eluates were separated by centrifuging the Sephadex and the fractions were examined for specific immunoglobulin by immunoelectrophoresis and polyacrylamide gel electrophoresis.

Isolation of bovine secretory IgA and serum IgA

Bovine milk whey was concentrated approximately ten-fold by dialysis against 30 per cent polyethylene glycol, and 100 ml of concentrated whey was fractionated on Sephadex G-150 (80 × 6 cm). The eluates in the exclusion peak were pooled, concentrated and recycled on Sephadex G-200 (90 × 2·5 cm) before further chromatography of the exclusion fraction on DEAE cellulose. The fraction eluted with 0·1 M NaCl in 0·01 M phosphate buffer pH 7·6 contained 11S IgA characterized as previously described (Porter and Noakes, 1970). Purification of the component was effected by isoelectric electrofocusing.

Bovine serum IgA was isolated from the serum globulin fraction precipitated with 12 per cent Na_2SO_4 by chromatography on DEAE cellulose. The globulin fraction was applied to the column in 0·01 M phosphate buffer pH 7·6. A stepwise elution schedule using 0·02 M phosphate buffer pH 6·3, 0·05 M phosphate buffer pH 4·5 and 0·2 M phosphate buffer pH 4·5 was used. Serum IgA detected in fractions eluted with the final buffer was further purified by gel filtration on Sephadex G-200 and by isoelectric focusing.

Isolation of secretory component

Free secretory component was isolated from bovine milk whey as described by Porter (1971).

Quantitative estimation of immunoglobulins

Immunoglobulins were assayed by the radial immunodiffusion technique of Mancini, Carbonora and Heremans (1965).

Antisera for the immunoglobulins and secretory component were prepared in rabbits by injection of emulsions in Freund's incomplete bactoadjuvant (Difco). The antisera were absorbed with precolostral calf serum and the non-specific immunoglobulins. Rabbit antisera were also prepared against bovine serum and milk-whey.

Bacterial antibody tests

Indirect haemagglutination and antiglobulin haemagglutination tests were carried out using sheep red cells modified with 08 lipopolysaccharide obtained from a haemolytic strain of *E. coli*. This is known to be one of the prevalent O groups demonstrated in calves dead from *E. coli* scepticaemia (Sojka, 1965).

Antiglobulin haemagglutination tests were carried out with specific antisera for bovine α, μ and γ chain in a manner similar to that described by Coombs, Jonas, Lachmann and Feinstein (1965).

Serum and lacteal secretions

Samples of colostrum were obtained at parturition from five Ayrshire cows and the calves were bled from the jugular vein at birth and 2–3 days later.

Whey from colostrum, milk and dry secretions was separated using the technique of Bohren and Wenner (1961).

RESULTS

ABSORPTION OF COLOSTRAL IMMUNOGLOBULINS AND *E. coli* ANTIBODIES BY THE NEONATAL CALF

The lack of prepartum transfer of immunoglobulins to the calves under investigation was examined by immunoelectrophoresis of sera taken at birth. The immunoelectrophoretograms were developed with immunoglobulin class specific antisera and in each case the calf sera were negative for IgA and IgM although traces of IgG were detectable. The considerable absorption of colostral immunoglobulins by calves is shown in Table 1

TABLE 1

IMMUNOGLOBULIN AND *E. coli* ANTIBODY LEVELS IN BOVINE COLOSTRUM AND THE SERUM OF SUCKLING CALVES

Sample	Immunoglobulin concentration (mg/100 ml)				*E. coli* 08 Haemagglutination antiglobulin titre		
	G1	G2	A	M	G	A	M
Molly							
Colostrum	7360	111	480	1280	2560	1280	2560
Calf serum	2600	40	144	410	640	320	640
Silver							
Colostrum	12400	300	380	1340	5120	320	2500
Calf serum	6000	180	176	580	1280	160	1280
Pansy							
Colostrum	8280	260	250	580	640	640	640
Calf serum	4420	225	192	230	320	640	320
Linda							
Colostrum	8960	325	320	780	20480	5120	10240
Calf serum	3840	135	164	240	10240	2560	5120
Daffodil							
Colostrum	7120	ND	270	610	640	640	640
Calf serum	4720	178	176	290	320	640	320

in which assays are recorded for calf sera taken at 2–3 days of age. The proportion of each immunoglobulin class in calf serum, matches fairly closely the proportion assayed in the related colostrum. This suggests a lack of selection in intestinal absorption of colostral immunoglobulins by the neonatal calf. This point is further substantiated by the gel filtration studies recorded later in the text.

The concentration of IgG2 in colostrum and calf serum was uniformly low. This finding was suggested by the observations of numerous other authors although no precise measurements were made, and has been interpreted as showing that a preferential transport of IgG1 rather than IgG2 occurs in the bovine mammary gland (Murphy, Aalund, Osebald and Carrol, 1964). However, since the concentration of IgA and IgM also exceeds that of IgG2 it is clear that selective transport may not be limited to IgG1. On the other hand it is uncertain how much of the individual immunoglobulins may be attributed to local synthesis in the bovine mammary gland rather than transport from the blood circulation of the cow across the mammary acinar epithelium.

Studies of *E. coli.* 08 antibody activity by antiglobulin haemagglutination with specific antisera showed in general a fairly even distribution of activity between the three immunoglobulin classes in both colostrum and calf serum. This suggested that secretory 11S IgA may have a role to play as a circulating antibody in the neonatal calf. This would be a

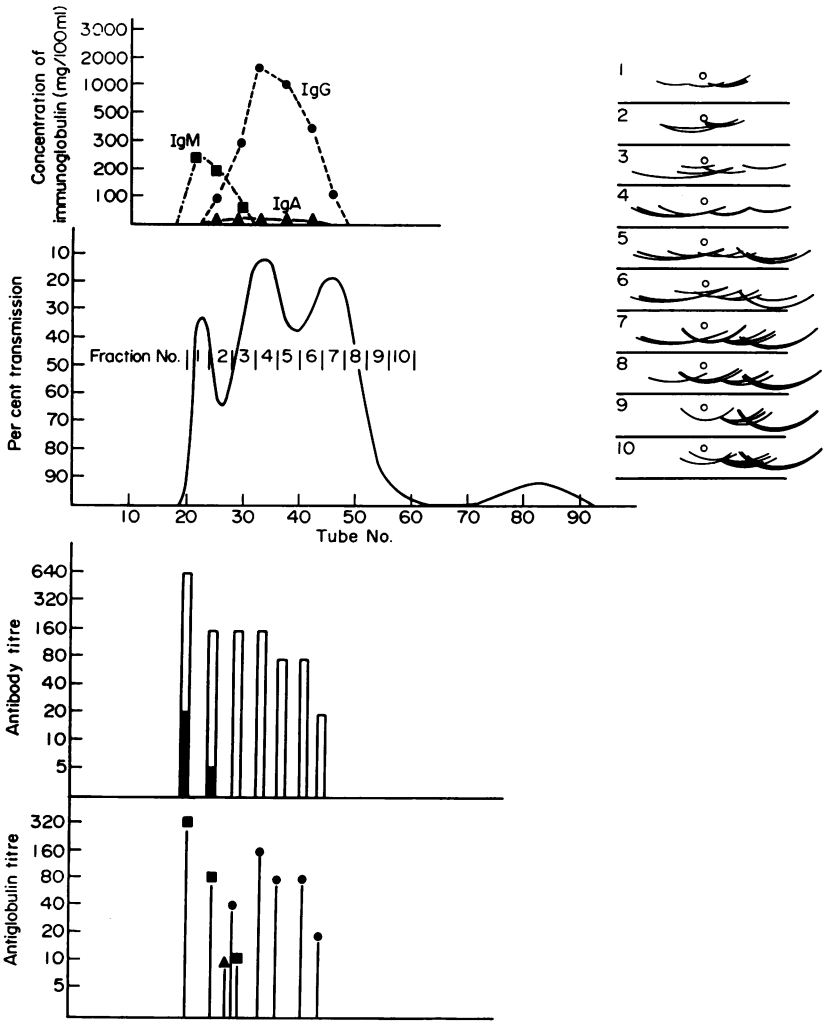

FIG. 1. Gel filtration of bovine serum on Sephadex G-200 giving pooling data, immunoelectrophoresis of ten selected fractions, elution characteristics of immunoglobulins assayed by radial immunodiffusion, *E. coli* 08 antibodies detected by haemagglutination and antiglobulin haemagglutination. ■, IgM; ▲, IgA; ●, IgG. Solid bars, *E. coli* 08 haemagglutination; open bars, *E. coli* 08 antiglobulin.

unique situation in the many species examined up to now and the observation was examined in more detail by gel filtration.

Chromatographic studies on Sephadex G-200 of cow serum, colostrum and 3-day calf serum are shown in Figs 1, 2 and 3. In each figure the elution characteristics of IgA, IgM and IgG are shown together with the assays of *E. coli* 08 antibody activities and the specific antiglobulin activity attributable to specific immunoglobulin classes.

Immunoglobulin-A in adult bovine serum was eluted predominantly in the second peak of the protein profile and only low concentrations were found. Little or no antibody activity was detected in the IgA class and the antibody was attributable mainly to IgM and IgG. In the gel filtration profile of bovine colostral whey, IgA was eluted mainly in the

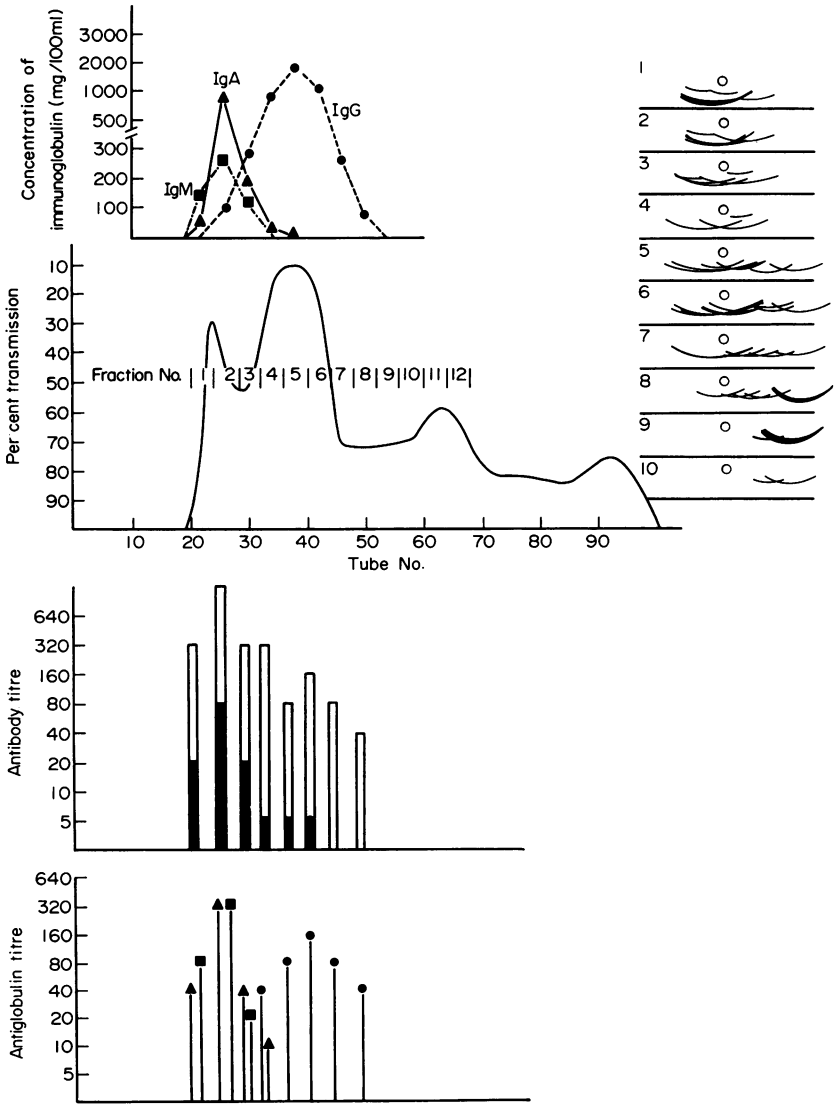

FIG. 2. Gel filtration of bovine colostral whey on Sephadex G-200; legend as for Fig. 1.

exclusion peak. This was immediately indicative of the difference in molecular size characteristics between normal adult serum IgA and that secreted in the colostrum. In contrast to serum, a high proportion of the *E. coli* 08 antiglobulin antibody in colostrum was attributable to the IgA class. In fact the activity due to IgA and IgM in the early gel

filtration fractions closely match each other. This was to be expected from the more general findings in Table 1.

The elution profiles of immunoglobulins and *E. coli* antibodies in gel filtration fractions of post-colostral calf serum (Fig. 3) were closely similar to those described for colostrum

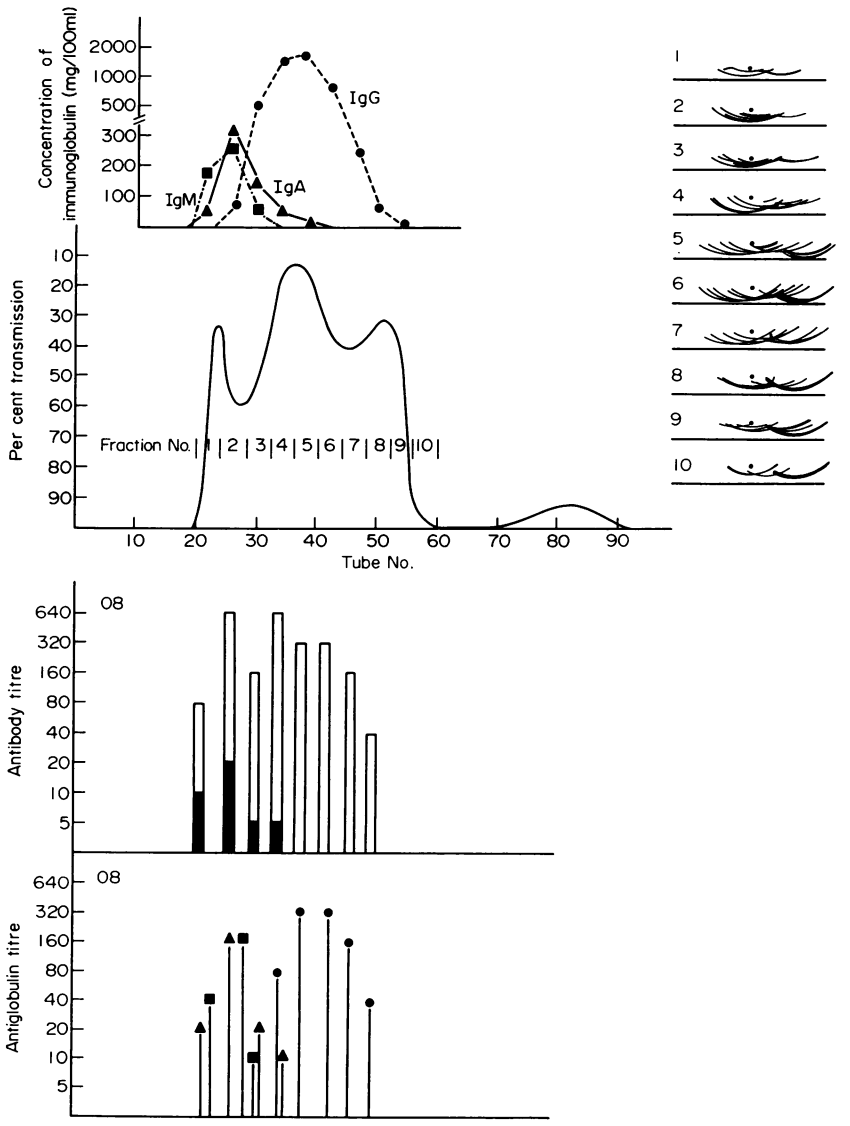

FIG. 3. Gel filtration of post-colostral calf serum on Sephadex G-200; legend as for Fig. 1.

in Fig. 2. This further confirmed the lack of selectivity in the calf for absorption of colostral immunoglobulins and also showed that large amounts of the 11S form of IgA-binding secretory component was present in calf serum. The antiglobulin test for *E. coli* was repeated using a rabbit antiserum for bovine secretory component and the activity

measured in fractions containing IgA was similar to that obtained with anti-α-chain. Thus the neonatal calf provides a unique example in which secretory IgA antibody is present in high levels in the blood.

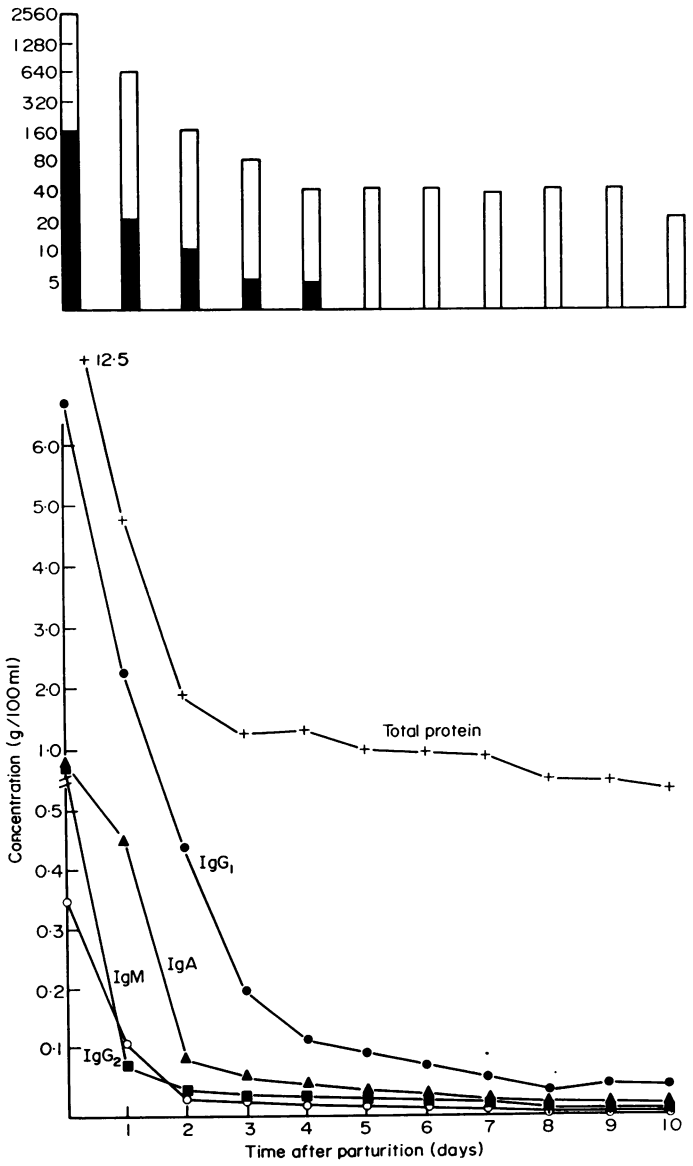

FIG. 4. Variation in immunoglobulins and *E. coli* 08 antibodies in bovine mammary secretions in early lactation. Open bars, *E. coli* 08 antiglobulin; solid bars, *E. coli* 08 haemagglutination.

IMMUNOGLOBULIN CHANGES IN BOVINE MILK AND CALF SERUM

The protein concentration of bovine milk whey declines very rapidly in early lactation falling to approximately 5 per cent of the early colostrum value within the first week.

This is due largely to a rapid decline in the concentration of secreted immunoglobulins and each immunoglobulin class is present in uniformly low amounts at the end of this period (Fig. 4). This is associated with an obvious decline in the level of antibody and only very low titres could be detected by the antiglobulin test.

The relationships between immunoglobulins and antibody in milk whey at 10 days post-

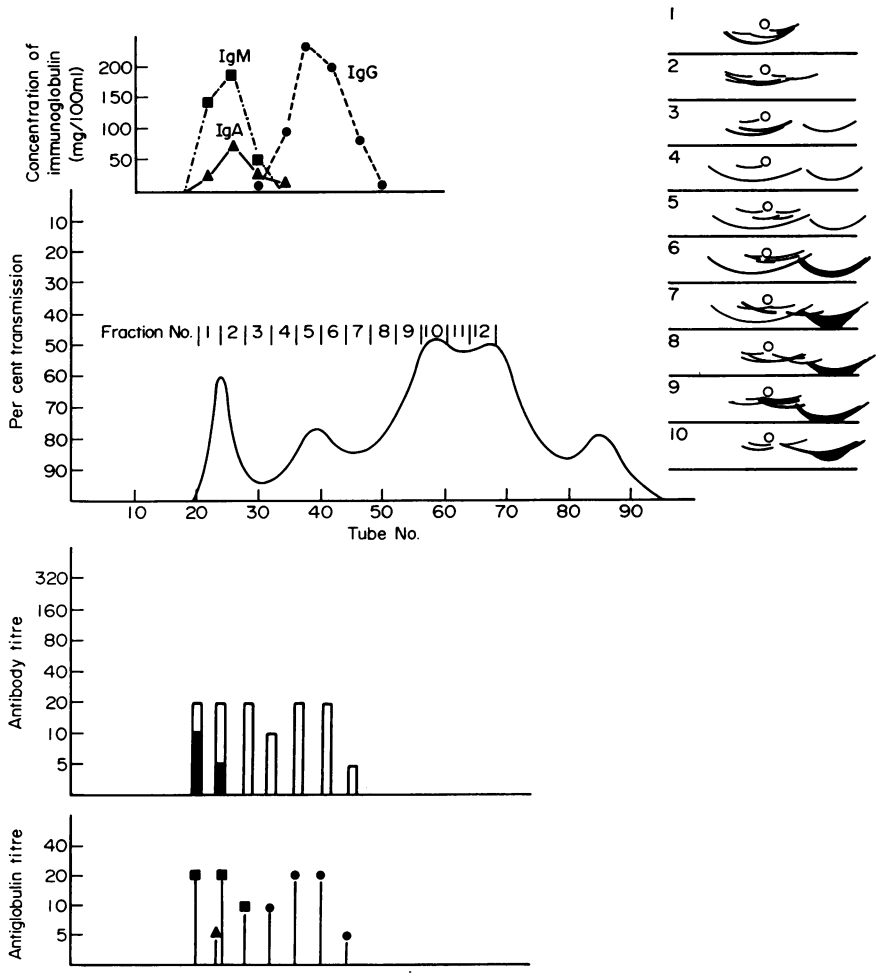

FIG. 5. Gel filtration of bovine milk whey on Sephadex G-200; legend as for Fig. 1.

partum were studied by gel filtration on Sephadex G-200 (Fig. 5). Very little activity was attributable to IgA and the low level of antibody was found to be distributed almost equally between the IgM and IgG fractions. Clearly immunoglobulins in bovine milk can have little significance in the local defence of the alimentary tract of the calf.

The transport of maternal antibodies by the colostrum and their persistence in the blood of the calf seems therefore to be of major importance in immunological defence. However, little is known about the persistence of passively acquired immunoglobulins in the calf. Periodic assays in calves over the first 6 weeks of life showed that the level of IgG declined

slowly and took more than 20 days to fall to less than half of the early post-colostral value. However, IgA and IgM showed a rapid fall in serum concentration (Figs 6 and 7) with half lives of approximately 2 and 4 days respectively.

DISCUSSION

Susceptibility to neonatal septicaemia caused by *E. coli* in the calf has generally been related to a deficiency in absorption of colostral antibody (Gay, Anderson, Fisher and

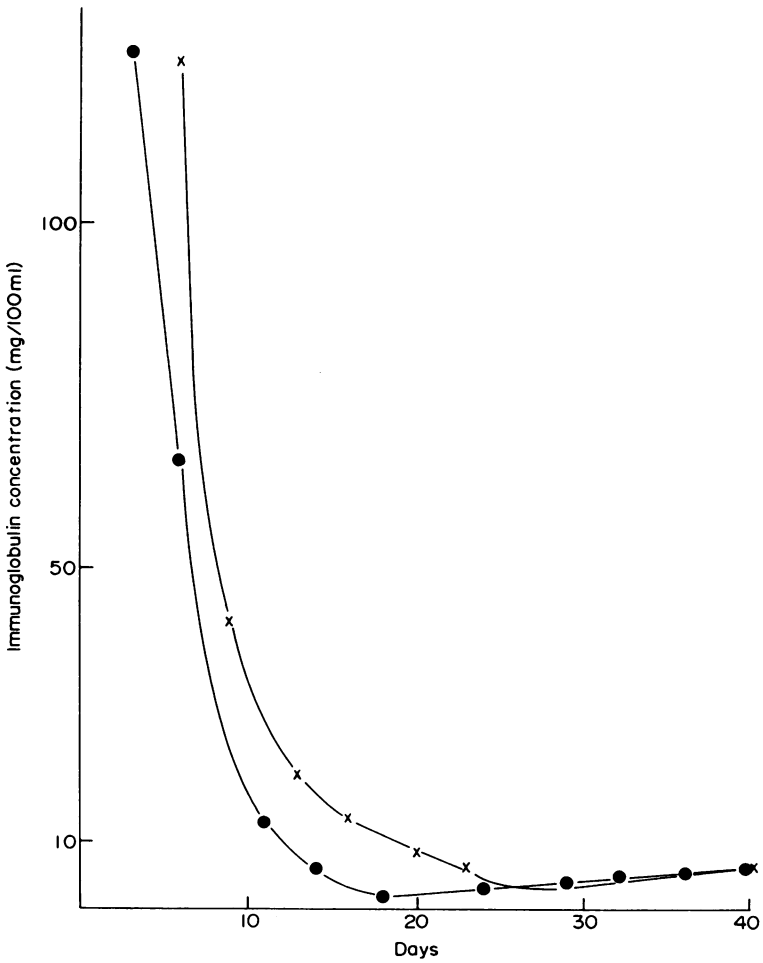

FIG. 6. Variation in serum levels of IgA in two colostrum fed calves.

McEwan, 1965). Until recently little effort had been made to define the nature of the protective antibody and most studies measured only the total γ-globulin. The relative transfer of immunoglobulin IgG1 to the colostrum and its absorption by the neonatal calf is quantitatively the outstanding feature of bovine immunology. However, functional

significance may not be related to abundance and it is necessary to assess the activity attributable to the different immunoglobulin classes.

Gay (1971) has proposed that the antiglobulin antibody activity to *E. coli* may have a considerable importance in neonatal defence. A correlation exists between the level of incomplete antibody and susceptibility to *E. coli* septicaemia and there was a universal

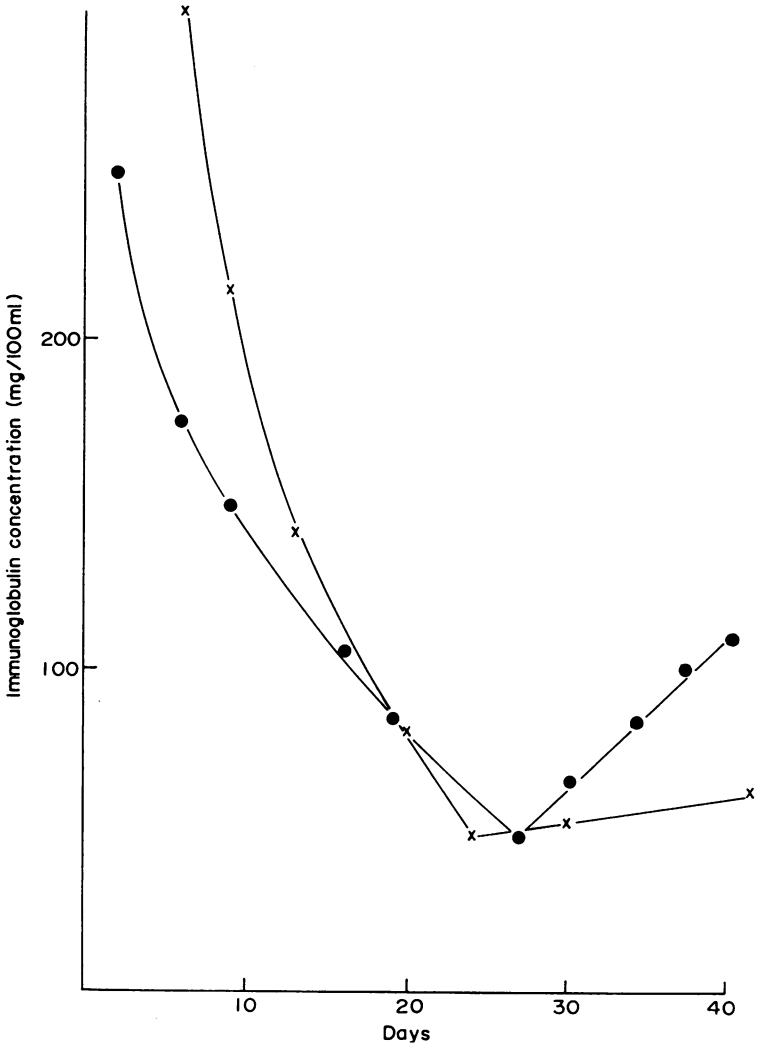

FIG. 7. Variation in serum levels of IgM in two colostrum fed calves.

association between death or survival and the absence or presence of incomplete antibody. Furthermore in studies of Ingram and Malcomson (1970) this type of antibody also appeared in the serum at an age when calves become resistant to *E. coli* septicaemia. The present studies have particular relevance to this postulate. Although IgA and IgM jointly account for less than 20 per cent of the total immunoglobulin in bovine colostral whey and post-colostral calf serum, they acquire a particular significance in this concept

since they each make a major contribution as antibodies to *E. coli* assessed by the anti-globulin.

Logan and Penhale (1971) have examined the capacity of IgG and IgM to protect neonatal colostrum-deprived calves against *E. coli* septicaemia. Colostral whey, or immunoglobulin fractions from it were injected intraperitoneally prior to challenge with a pathogenic strain of *E. coli*. Protection could be obtained with colostral whey but none was provided by either immunoglobulin fraction. The failure of IgM to provide protection was surprising in view of the *in vitro* activity against *E. coli* and the authors were compelled to considered the possibility of other protective factors in addition to these immuno-globulins.

The immunoglobulin profile and distribution of *E. coli* antibodies in post-colostral calf serum is very similar to that found in colostrum. The presence of high levels of secretory IgA in the serum of the neonatal calf is a finding not recorded for any of the numerous species in which a secretory IgA system has been identified. Thompson, Asquith and Cooke (1969) have demonstrated the presence of low levels of secretory IgA in the serum of patients with intestinal disease. This was probably due to transmission of intestinal secretory IgA across a damaged epithelium. In the pig there is little or no absorption of secretory IgA *E. coli* antibodies from the colostrum (Porter, 1969), which is quite in contrast to present findings in the calf. From the teleological standpoint this unique yet normal situation in the neonatal calf which provides high concentrations of secretory IgA *E. coli* antibody in the blood must have considerable importance in neonatal defence. Possibly this is the additional protective factor in colostrum which was lacking in the studies of Logan and Penhale (1971).

The level of IgA in the serum of the calf declines rapidly during the first week of life. The half life of approximately 2 days is very similar to that found by Butler, Rossen and Waldman (1967) for intravenously injected 11S IgA in man. The short half life of colostral IgA in the serum of the calf may seem to militate against its effective action in defence, but the manner in which it is lost from the circulation is possibly the means by which it contributes effectively to protection. We have studied extracts of faeces over the first 2 weeks of life and have preliminary evidence of a steadily declining excretion of antibody which is mainly attributable to IgA. This possibly shows that the intestine is a site for loss of circulating secretory IgA which would be quite distinct from the active process of synthesis and secretion of IgA in the intestinal mucosa. This latter process has now been demonstrated in the small intestinal tissue of the calf (Porter, Noakes and Allen, 1972), and under normal circumstances of development probably begins to operate as a host defence mechanism early in the second week of life.

The ineffectiveness of IgM antibodies in the experiments of Logan and Penhale (1971) may be related to the inability of this large molecule to pass from the circulation and penetrate the tissues to deal effectively with an invading bacterium at the level of the intestinal mucosa. IgA disappears from circulation at more than twice the rate of IgM and its capacity to re-enter the intestinal lumen after the first colostral absorptive phase may well be a factor contributing to effective local defence provided by a passively acquired antibody.

In bovine mammary secretions there is a rapid fall in immunoglobulin content so that after a few days all immunoglobulins and antibody activity have declined to very low levels. This again is in contrast to findings in the pig in which IgA plays a major role as an *E. coli* antibody throughout lactation (Porter, Noakes and Allen, 1970), and probably

makes a major contribution to local intestinal defence in the young pig. Clearly milk immunoglobulins must contribute very little to intestinal protection in the calf.

A wide range of antibacterial antibodies including *E. coli* antibodies have been demonstrated in bovine dry udder secretions (Reiter and Oram, 1967) and the mammary gland has been shown to respond to local stimulation with secretion of antibody (Sarwar, Campbell and Peterson, 1964). Up to now there have been no recorded studies of the nature of the bovine antibody, although it has been shown that infusion of the sheep mammary gland with Salmonella antigens gives rise to antibody in the milk which is mainly attributable to an immunoglobulin analogous to IgA (McDowell and Lascelles, 1969). Thus the present findings that secretory IgA is a major *E. coli* antibody in bovine colostrum provides material for interesting speculation on the role of this immunoglobulin in mammary defence. Although it is quantitatively less important than IgG1 it is probable that it is produced locally rather than having its origin in the serum. This has yet to be established in the bovine, but it is becoming clear that IgA plays a major role in local immune synthesis in other tissues (Mach and Pahud, 1971; Butler 1971; Porter, Noakes and Allen, 1972).

There is an interesting conflict in histological observations of the bovine udder in relation to its potential for cellular immune synthesis. Immunofluorescent studies provided no evidence of immunoglobulin containing cells at any time during lactation and suggested that immunoglobulins in mammary secretions were entirely derived by transport across the cells of the mammary acinar epithelium (Dixon, Weigle and Vazquez, 1961; Feldman, 1961). On the other hand earlier studies by Campbell, Porter and Petersen (1950) had provided evidence for plasmacytosis in the bovine udder during the period of colostrum formation. This suggests a potential for a local immune response which was confirmed later by Sarwar *et al.* (1964). The present observation of high concentrations of IgA with *E. coli* antibody activity in colostrum apparently quite unrelated to that found in serum would lend further support to the observations of Campbell *et al.* (1950). Clearly the findings of Dixon *et al.* (1961) should be re-examined with the use of specific immunoglobulin fluorescent antisera.

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